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Review

Effectiveness of 3-dimensional analysis of ambulatory motor function during the process of artificial nerve regeneration in dogs

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Abstract

We longitudinally assessed mainly ambulatory motor function using beagles with nerve regeneration via a polyglycolic acid (PGA)-collagen tube. Histological and electrophysiological assessment are important means of assessment of the regeneration process, although the ultimate goal of nerve regeneration is the recovery of motor function, so nerve regeneration is effective when this recovery is first noted.

We 3-dimensionally analyzed motor function assessment and studied techniques for 3-dimensional analysis of ambulatory motor function during nerve regeneration. The result was that 3-dimensional motor function assessment was useful for ambulatory motor function assessment of nerve regeneration via a PGA-collagen tube.

Key words: nerve regeneration, PGA-collagen tube, motor function assessment

1 Introduction

Treatment of peripheral nerve damage is a major theme in orthopedic surgery. Autologous nerve grafting is clinically the first choice as a method of reconstructing defects in peripheral nerves not allowing direct anastomosis for peripheral nerve damage due to a variety of causes. However, there are limits to nerves that can be selected for grafting, and loss of function for the area controlled by the nerve selected becomes a problem. In contrast, artificial nerves have the advantage of not requiring consideration of quantitative limits for grafting and loss of function for the site of the nerve selected.

Thus, the current authors developed artificial nerves and studied their usefulness [1–4]. In addition, means such as morphological observation, electrophysiological measurement, tracking of axonal transport using a tracer, and observation of ambulatory patterns were used as methods of determining artificial nerve regeneration. Given, however, that as long as the regeneration of action, i.e., an ambulatory pattern, is not obtained, then the result cannot be labeled true regeneration even if results of regeneration are noted morphologically and electrophysiologically, so detailed analysis techniques for ambulatory patterns are needed. Nonetheless, methods of assessing ambulatory patterns are principally

behavioral assessment and video assessment [5–7], and there are few reports that have analyzed ambulatory patterns in detail [8].

The current research reports on compensating for 80–mm defects in canine peroneal nerves using a fiber-filled artificial nerve tube and performing long-term assessment of ambulatory motor function in 3 dimensions primarily for functional assessment of the regenerated nerves.

2 Materials and Methods

2. 1. Polyglycolic acid (PGA)-collagen tube

An external tube of PGA mesh with an inner dia. of 4mm and length of 90mm with a wall thickness of 50μm twisted in a tube shape had an amorphous collagen coating added to both sides. About 80 collagen fibers with a dia. of 50μm and length of 90mm were coated with 10μg/ml laminin and placed inside (Fig. 1). Types I + III atelocollagen extracted from pig skin by enzyme treatment were used for the collagen, and thermal dehydration cross-linking was done for 24 hrs at 140℃ to delay the in vivo rate of absorption.

2. 2. Surgical procedure

Subjects were placed under general anesthesia with pentobarbital sodium (Nembutal) (30–50mg/Kg). The left peroneal nerve of 12 of 13 adult beagles (body weight 8–15kg) was resected 80mm, the peroneal nerve was inserted to a depth of about 5mm into a PGA-collagen tube so as to leave a gap of 80mm between both ends of the nerve stumps, and the epineurium and tube were fixed by sutures using non-absorbent thread (Fig. 2). In addition, the adult beagle that underwent no surgery at all served as the normal control.

2. 3. Macroscopic and histological assessment

Twelve months after surgery, systemic perfusion fixation was performed with 1% glutaraldehyde in 0.1M phosphate buffer solution (pH7.4) under general anesthesia, and specimens were removed and observed. Afterwards, prefixation was performed with the same solution and postfixation was performed with 2% osmic acid in 0.1M phosphate buffer solution (pH7.4); specimens were then embedded in resin. Thinly sliced specimens of 1–μm were stained with toluidine blue and observed with an optical microscope.

2. 4. Electrophysiological assessment

After intramuscular injection of ketamine (5mg/100g), pentobarbital sodium (Nembutal) (1mg/100g/h) was administered intraperitoneally and the postoperative course (14, 18, and 48 months postoperatively) was observed using the Nicolet Viking Quest System (Nicolet Biomedical Inc., Madison, WI, US). The sciatic nerve at the sciatic notch was electrically stimulated and the compound muscle action potential (CMAP) of the anterior tibial muscle was recorded as an index of the recovery of peripheral nerve fibers. In addition, the anterior tibial branch of the peroneal nerve was stimulated and somatosensory

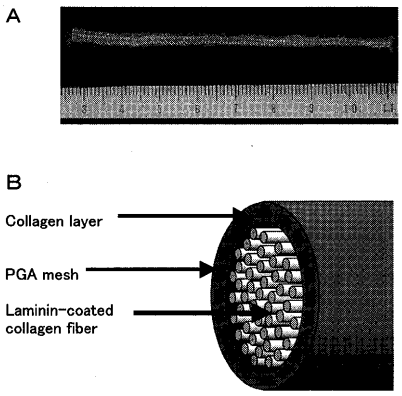


Fig. 1 Illustration of (A) macroscopic appearance (outward), (B) the structure

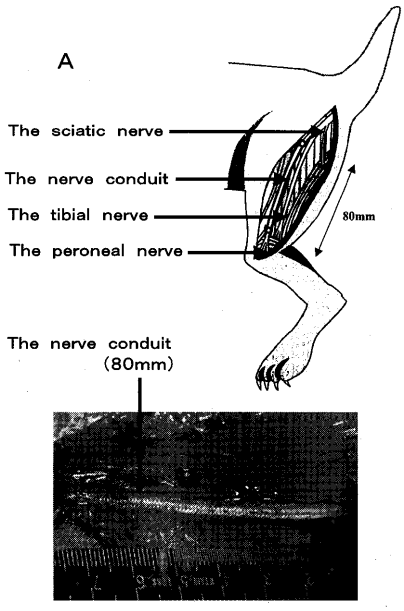


Fig. 2 Experimental procedures of (A) implanted dog and intra-operative view after implantation of the nerve conduit

evoked potentials (SEP) in the sensory cortex were recorded as an index of the recovery of the sensory nerve system overall.

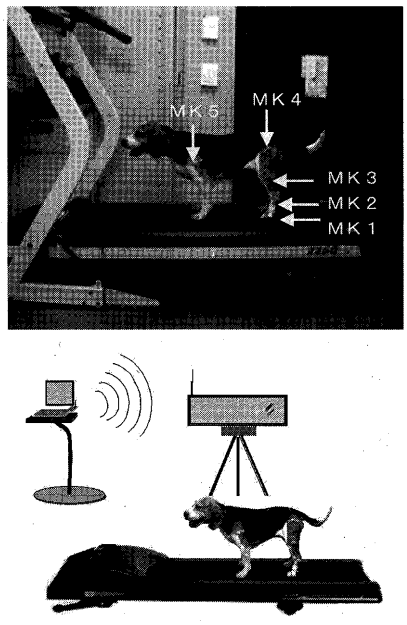
2. 5. Measurement of ambulatory motion

To measure articular movement at 14, 18, and 48 months after compensation with the PGA-collagen tube, markers (MK5 : acromion, MK4 : greater trochanter, MK3 : knee, MK2 : external condyle, and MK1 : head of the fifth metatarsal bone) were attached on the side with artificial neural compensation, and loaded running was measured (Fig. 3) with a UM-CAT 3-dimensional motion analysis system (Unimec, Tokyo, Japan) on a UM-22510 treadmill (Unimec, Tokyo, Japan). A 3-dimensional coordinate plane was determined from the movement of individual markers, with MK5/MK4/MK3 (hip joint angle), MK4/MK3/MK2 (knee joint angle), and MK3/MK2/MK1 (ankle joint angle). In addition, the belt speed of the treadmill was set at 4km, and three types of loaded running — with no inclination or declination (0°), with a 4° decline (−4°), and with a 4° incline (+4°) — were done. For measurement, the beagle was placed on the treadmill and, the 3 seconds from when the belt speed reached 4 km were recorded. The timing when maximum extension of individual joint angles (hip joint, knee joint, and ankle joint angles) occurred was determined as a parameter from the records.

2. 6. Analysis of results of ambulatory motion measurement

To calculate parameters to quantify/convert the degree of improvement in motor function to numerical values, a timing chart with timing when maximum extension occurred as ON and maximum flexion timing as OFF was created based on time-series data for individual joint angles (hip joint, knee joint, and ankle joint angles) (Fig. 4).

The period from ON in the timing chart for the hip joint to the next ON was defined as the base period, and the timing when maximum extension of the knee joint and ankle joint occurred was determined as a parameter (Pk



(A) : A figure of a marker position.
MK5 : Acromion (Shoulder),
MK4 : Greater trochanter (Hip),
MK3 : Lateral epicondyle of the femoral (knee),
MK2 : Lateral malleolus of the fibla (Ankle),
MK1 : Basis of the fifth metatarsal bone
(B) : A figure of measurement outline.

Fig. 3 Walking movement instrumentation

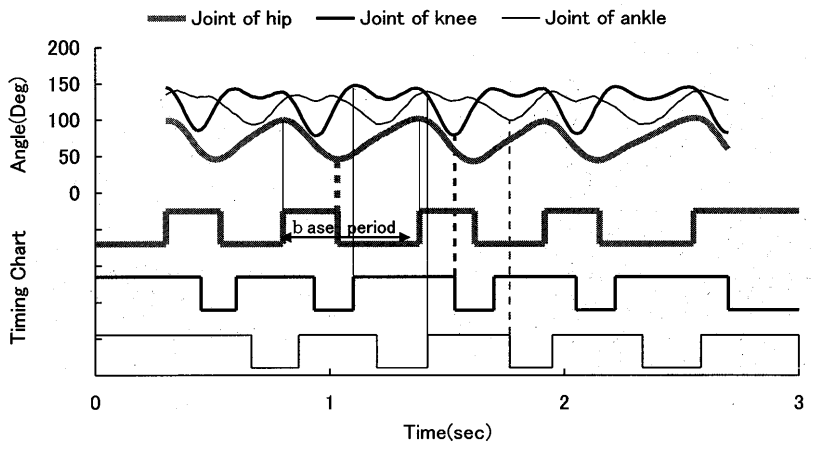


Fig. 4 The maximal extension and the maximal flexure timing graph (14M)

$$Pa = \frac{Ta-Ts}{Te-Ts}\times 100 \qquad Pk = \frac{Tk-Ts}{Te-Ts}\times 100$$

Fig. 5 An equation of timing parameter

and Pa respectively). Variables required for calculation of parameters were *Ts* and *Te* as the start and end time for the base period (*Ts* was the time when maximum extension of the hip joint occurred, and *Te* was the tine when the next maximum extension of the hip joint occurred), *Tk* as the time when maximum extension of the knee joint occurred, and *Ta* as the time when maximum extension of the ankle joint occurred, and parameters were calculated (Fig. 5). However, the range of analysis covered 4 base periods, so parameters (Pa and Pk) that calculated each of the 4 base periods were averaged in this analysis.

The aforementioned animal experiments were conducted in accordance with Kyoto University Animal Experimentation Guidelines (1989).

3 Results

3. 1. Findings from macroscopic and histological assessment (Fig. 6 and 7)

In terms of macroscopic findings, comparison with the normal control nerve indicated that regeneration did morphologically occur although the morphology was not uniform. In terms of optical microscope findings from toluidine blue staining of individual regenerated nerves 12 months after surgery and the normal control nerve, regeneration of numerous myelinated nerves was noted. However, regenerated nerves had a smaller axonal diameter overall compared to the normal nerve and the thickness of the myelin sheath tended to be thinner. This tendency was marked in more peripheral portions of the regenerated nerves.

3. 2. Electrophysiological findings (Fig. 8)

With regard to electrophysiological assessment, evocation of the CMAP was noted in some of the dogs 2 months after surgery and was noted in most of the animals in the 12th month. In addition, SEP was also noted in most of the animals in the 12th month.

3. 3. Ambulatory motion analysis findings

There were differences in ambulatory motion for healthy legs and the prosthetic leg of the resected lower limb; these differences were largely attributed to muscle strength. When humans and animals run, they use muscles, control individual joints, and maintain a gait (form), although when one has a prosthetic leg this process depends on the moment of inertia of the prosthetic leg, so differences between the two in related motion of individual joints is readily surmised to occur.

Looking at the current parameters (Pa and

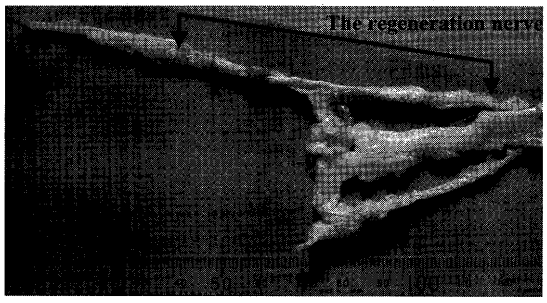
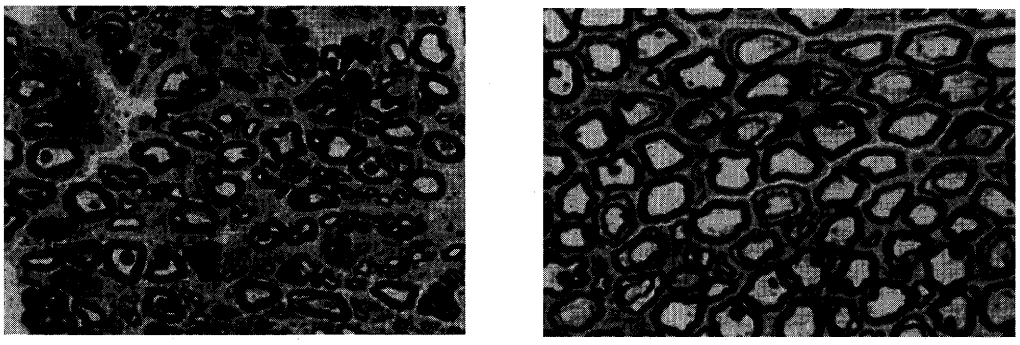


Fig. 6 The regeneration nerve which was taken out



Regenerated segment　　Normal nerve

Fig. 7 Regeneration nerve part for postoperative 12 months and optical microscope findings of toluidine blue stain of normal nerve

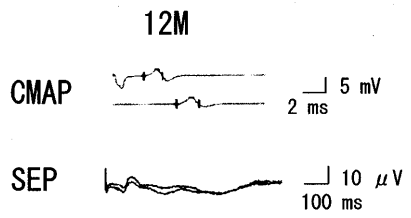


Fig. 8 CMAP after 12 months and SEP

Table 1 A timing parameter

	14M	18M	48M	Normal
Pk(%)	60	59.3	51.5	44.4
Pa(%)	113.4	107.6	45.5	40.9

Pk) indicates that, in proportion to the postoperative course, Pk (timing parameter for maximum extension of the knee joint) and Pa (timing parameter for maximum extension of the ankle joint) were confirmed to approximate normal parameters (Table 1).

In addition, drawing a regression line for Pa indicated that Pa=0% at about 50 months after surgery, with almost the same timing as the normal dog (Fig. 9). Thus, motor function is gradually recovered in several months after transplant of the artificial nerve tube and is surmised to be recovered to a level just like that of the normal dog in about 50 months. The result of nerve regeneration, although indirect, is that motor function is recovered.

4 Discussion

Functional assessment of the process of artificial nerve regeneration is principally observation with a video camera [5–7], although given that the ultimate goal of regeneration via artificial nerves is, without exaggerating, the restoration of motor function, more detailed behavior assessment is needed as a recovery index. With regard to peripheral nerve regeneration in particular, muscles controlled by the regenerated nerve must function in cooperation while the individual walks, so electrophysiological assessment is a must and assessment of 3-dimensional movement of individual joints that comprise motion is needed.

The current research into longitudinally assessing a model that provided compensation with a PGA-collagen tube indicated that, regardless of in vivo macroscopic findings and histological and electrophysiological regeneration, ambulatory motor function was not completely restored.

Various factors are thought to be the cause of this; one is thought to be because adaptive control was not working well [11, 12]. Adaptive control is originally supposed to be response to target input that is kept constant regardless of the environment and is reflected in differentiation of motion patterns. Differentiation in motion patterns is markedly seen particularly in ambulatory motion, and motion patterns for ambulatory motion can, as an ambulatory pattern, primarily be characterized as a phase difference for leg motion. Even with the current model, adaptation to factors for environmental changes such as speed and inclination/declination is difficult depending on the postoperative period, and deviation sin ambulatory timing appear.

With ambulatory motion like this, control of the path of individual leg motion is a crucial problem. Coordination between several legs is

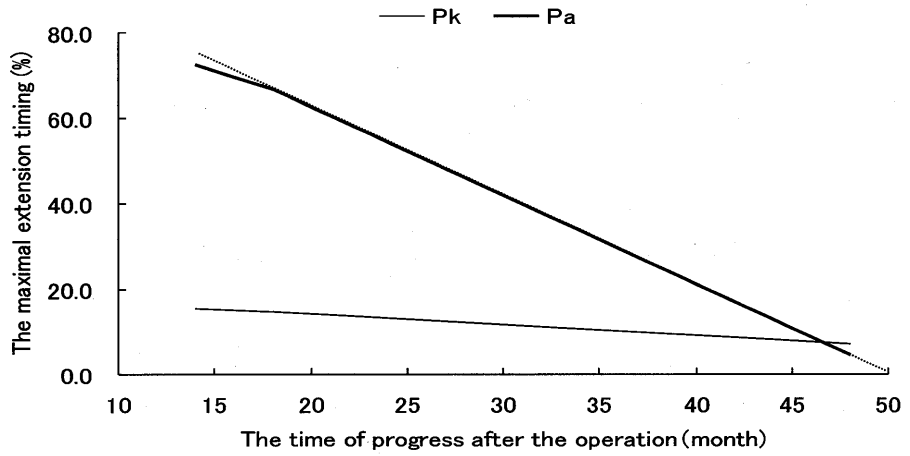


Fig. 9 The maximal extension timing graph which turned a normal dog into

required to achieve stable ambulation. This coordination manifests as the rhythm of ambulation and forms ambulatory patterns. That is, ascertaining ambulatory patterns becomes vital to the phenomenon of recovery of ambulatory motor function.

Here, a discussion will be presented based on analysis results for ambulatory patterns as the current authors attempted. In the current analysis, the differential delay from the time when maximum extension of the hip joint occurred until the time when maximum extension of the knee joint and ankle joint occurred was selected as a parameter (Pa, Pk). Based on the current experiment, there is almost no difference in parameters (Pa and Pk) for a normal dog (Fig. 10), and a major difference occurs for a dog 14 months after surgery (Fig. 11). This difference is attributed to the anterior tibial muscle not working and the individual being unable to control plantar flexion/dorsal flexion because there is insufficient nerve regeneration with the PGA-collagen tube grafted for the resected left peroneal nerve.

Thus, the foot for which plantar flexion/dorsal flexion cannot be controlled is assumed to be equivalent to pendular movement of a rigid body. The presence or absence of muscle strength control according to timing when maximum extension/maximum flexion of individual joints occurs is discussed through analysis of pendular movement via a fixed-body model.

Conditions for the analysis of pendular movement were the length r and mass m of the foot and length 2r and mass 2m of the lower leg as rigid bodies (Fig. 12), and the angular

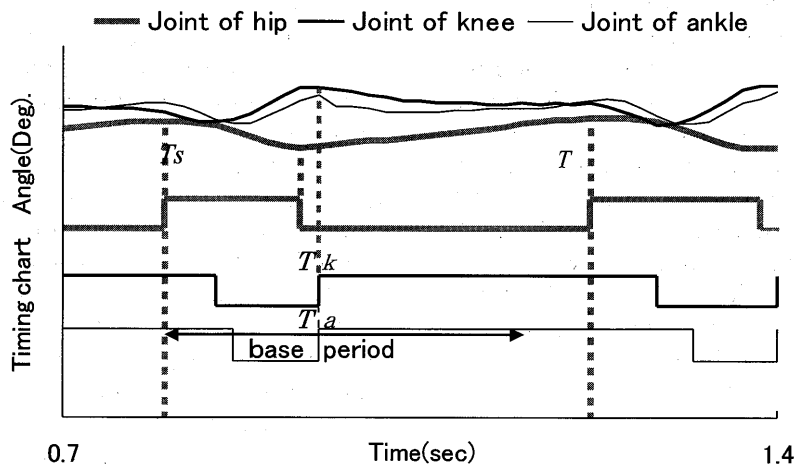


Fig. 10 The timing chart of joint angle cover base period (Normal)

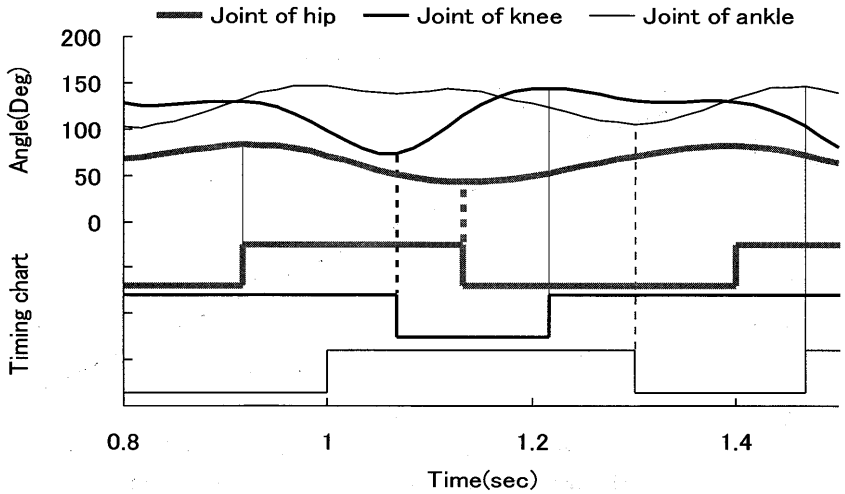


Fig. 11 The timing chart of joint angle cover base

velocity of the foot and lower leg was determined (Fig. 13). The angular velocity of the lower leg resulted in a $\sqrt{2}$ — fold delay compared to the foot. In addition, the thigh is raised with angular momentumτ. Displaying the instant when the foot leaves the floor (Fig. 14: difference of $\alpha 1$–$\alpha 2$ for the joint angle of the knee joint and ankle joint) as a time-series graph simulating the joint angle displacement of the knee joint and ankle joint confirmed that the phase and period width did not coincide in the foot model and lower leg model (Fig. 15). This occurred because of shifting of phases for angular displacement ($\alpha 1$ and $\alpha 2$) produced by variation in the angular velocity as part of pendular movement of rigid bodies

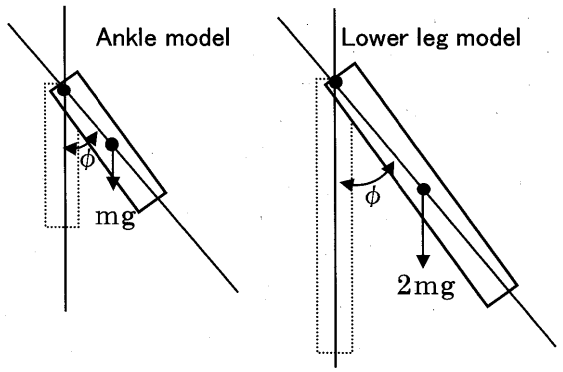


Fig. 12 Rigid Bodies

I : Moment of inertia
ω : Angular velocity
M : mass
L : Length
R : Length of ankle
2r: Length of lower leg
Rotational energy of about axis $= \frac{I\omega^2}{2}$
Potential energy of tilt angle ϕ $= \frac{r}{2}(1-\cos\Phi)Mg$
$I\omega^2 = L(1-\cos\Phi)Mg$
$I = \frac{L^2M}{3}$
$\omega^2 = 3(1-\cos\phi)\, g/L$
Angular velocity of ankle : $\omega_1{}^2 = 3(1-\cos\phi)g/r$
Angular velocity of lower leg : $\omega_2{}^2 = 3(1-\cos\phi)g/(2r)$
$\omega_1{}^2 = 2\omega_2{}^2$
$\omega_1 = \sqrt{2}\,\omega_2$

Fig. 13 The ratio of angular velocity of knee to angular velocity of ankle

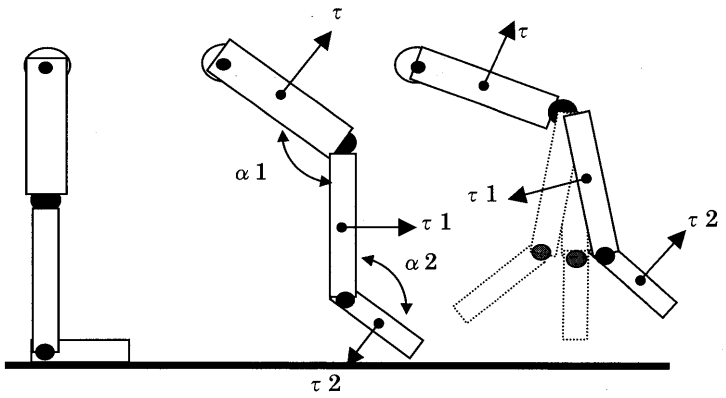


Fig. 14 Rigid Bodies of the lower extremity

with differing lengths.

Thus, a phase shift is thought to occur in the spatial joint angle alteration of the knee joint and ankle joint during running when muscle strength of the lower leg is not acting. Thus, the phase difference for spatial joint angle alteration of the knee joint and ankle joint was verified based on parameters (Pa and Pk) for a normal dog and post-operative dogs as obtained in the current experiment. With a normal dog, there is almost no difference in parameters (Pa and Pk), so the spatial joint angle for the knee joint and ankle joint was confirmed to be almost synchronous. Thus, a normal dog uses external forces other than gravity and controls the knee joint and ankle joint. Here, there are no external forces in addition to muscle strength, and a normal dog counters angular momentum produced by gravity, segment length, and the moment of inertia with muscle strength, controlling the spatial joint angle of the knee joint and ankle joint so that the angles are synchronized. In addition, neural pathways must be sound to control muscle activity.

A difference in parameters (Pa and Pk) occurred for dogs 14 months after surgery, so this confirmed that the spatial joint angle of the knee joint and ankle joint were not synchronized. Thus, muscle tone itself declined and contraction and extension were not possible, or neural pathways were not sound. In the current model, the left peroneal nerve was resected, so the left peroneal nerve had not been adequately regenerated. This fact was, although indirectly, confirmed by parameters (Pa and Pk) of dogs 48 months after surgery approximating those of a normal dog. In addition, nerve regeneration did take place proportional to the time after surgery (Fig. 9).

Considering motion to bring lower limbs forward as a cause of shifting of phases indicates that when the quadriceps femoris muscle is used and the lower leg is raised, muscle on which angular momentum is acting in the direction of extension or plantar flexion in the vicinity of the ankle joint, i.e., angular momentum to maintain balance, produces torque in the opposite direction. However, control is not possible when nerve regeneration is insufficient and the anterior tibial muscle, one of the muscles that controls plantar flexion/dorsal flexion of the ankle joint and that originally controls extension, is not working in cooperation. The result is that a shifting of phases for the spatial joint angle of the hip joint and knee joint occurs.

In addition, the current model confirmed that, regardless of whether the level of artificial neural compensation is at a site not directly affected by the hip joint or knee joint, the maximum extension timing for the knee joint with respect to the maximum extension timing for the hip joint of dogs after surgery was delayed in comparison to that of a normal dog (Table 1). This is surmised to be because of the need to adjust the hip joint and knee joint and prolong the swing phase. The most reasonable cause for the prolonged swing phase is use of the moment of inertia of the ankle joint by lengthening the swing phase to bring the foot that cannot be controlled by muscles forward before it scrapes the ground and action to flex from mid to late swing the ankle joint that contacts the floor. In actuality, maximum extension of the ankle joint is displayed when the lower limb is kicked out towards the rear, alteration into the maximum flexion is displayed from the end of the stance phase to start of the swing phase (Fig. 16).

Thus, when ankle joint control by muscles is not possible the moment of inertia is used for

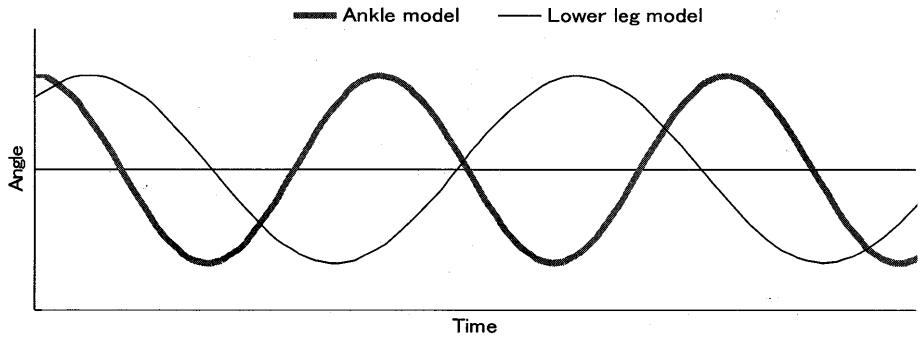


Fig. 15 Joint angle of model

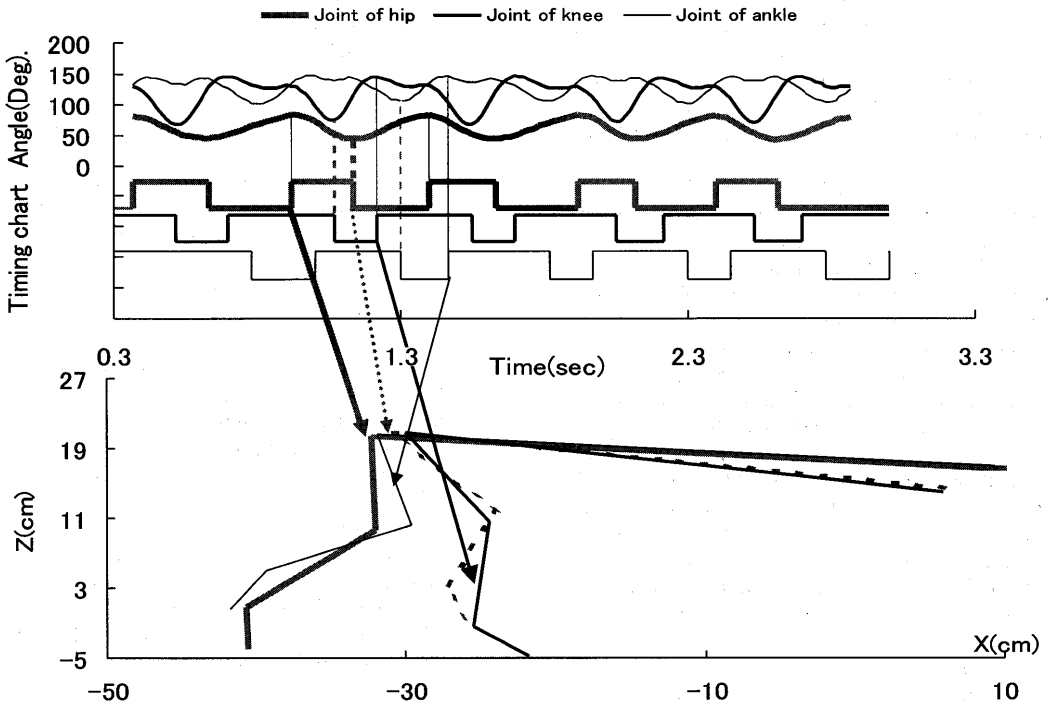


Fig. 16 The timing chart of joint angle and stick picture (14M)

extension/flexion motion of the ankle joint by adjusting the extension/flexion of the hip joint and knee joint. Thus, flexion/extension timing of the hip joint and knee joint that were not directly affected by artificial neural compensation was delayed in comparison to that of a normal dog.

Thus, developing parameters for motor function recovery to ascertain the process of nerve regeneration allows discernment of the muscle function controlled by the regenerated artificial nerve and discernment of motor function overall as a system. Perceiving individual sections as a system and reconstructing it as a whole are essential parts of ascertaining regeneration indices for function recovery, the ultimate goal of nerve regeneration. Therefore, 3-dimensional discernment of ambulatory patterns will serve as an index of motor function recovery for nerve regeneration and is just as important an assessment item as morphological and electrophysiological assessment. In the future, the current authors would like to effectively incorporate 3-dimensional motion analysis as a form of assessing function recovery.

5 Conclusion

The current research into longitudinally assessing a model that provided compensation with a PGA-collagen tube indicated that, regardless of in vivo macroscopic findings and histological and electrophysiological regeneration, ambulatory motor function was not completely restored. However, ambulation was performed even without complete restoration, i.e. adapting to the environment. In the future, attainment of ambulatory patterns close to normal ambulation is projected. What this projection allows is macroscopic, morphological, and electrophysiological assessment, which are results obtained by 3-dimensionally analyzing ambulatory motor function. Morphological recovery is crucial, although the original meaning of recovery is surely that the restored morphology works functionally.

In the future, various artificial organs will be morphologically restored, and restoration in terms of function in the environment inside and outside of the body will be crucial.

Acknowledgments

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